

Customer No.	026418	
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE		
Attorney's Docket No.:	GK-ZEI-3152 / 500343.20153	
U.S. Application No.:	10/049,548	
International Application No.:	PCT/EP01/07104	
International Filing Date:	JUNE 22, 2001	22 JUNE 2001
Priority Date Claimed:	JUNE 26, 2000	26 JUNE 2000
Title of Invention:	FLUORESCENCE MICROSCOPE	
Applicant(s) for (DO/EO/US):	Reiner MITZKUS and Alex SOELL	

ENGLISH  
TRANSLATION  
OF

Publication No. WO 02/01272

Published 03JAN02

Docket No.: GK-ZEI-3152/500343.20153

## FLUORESCENCE MICROSCOPE

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### CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority of PCT Application Serial  
No. PCT/EP01/07104 filed June 22, 2001 and German Application No.100 30 929.1  
filed June 26, 2000, the complete disclosures of which are hereby incorporated by  
10 reference.

### BACKGROUND OF THE INVENTION

#### a) Field of the Invention

15 The present invention relates to microscopes equipped for handling  
fluorescence applications.

#### b) Description of the Related Art

20 A known microscope for fluorescence applications is shown in Fig. 1.  
The beam path in a microscope equipped for fluorescence applications is shown in  
that figure.

The light from an additional light source (1) passes through a heat-  
absorbing filter (2), red attenuating filter/stop slide (3) and a field diaphragm (4) to  
the excitation filter (5). The latter is installed in the reflector slide of the microscope  
25 which also contains a dichroic beam splitter (6). The dichroic beam splitter reflects  
the shortwave excitation light through the objective (7) into the specimen or  
preparation (8).

The occurring emission is collected by the objective (7) and - because  
it has greater wavelengths than the excitation light - is passed by the dichroic beam  
30 splitter (6). The beams now pass through the emission filter (9). The remainder of  
the excitation light is filtered out by the latter. For this reason, this filter is also

referred to as a blocking filter. As is conventional, the tube lens (10) and eyepiece (11) form the microscope image formed of fluorescent light.

### The Problem Addressed by the Invention

In order to avoid image offset (pixel shift), multiple exposures in fluorescence recordings with different emission filter sets (A, B) require an optimal congruence of the object image in the individual recordings. However, there are technological limits in this respect.

Because of the different wedge angles of the emission filters ( $A_{EM}$ ,  $B_{EM}$ ) and of the color splitters, the filter combinations needed for the fluorescence application cause a slight image offset. This is shown in Fig. 2.

The reference symbols have the following meanings:

	$A_{EM}$	emission filter of filter set A
	$B_{EM}$	emission filter of filter set B
	$a_1$	light beam striking $A_{EM}$
	$b_1$	light beam striking $B_{EM}$
	$a_2$	light beam deflected by $A_{EM}$
	$b_2$	light beam deflected by $B_{EM}$
	$\alpha_A$	angle between the incident light beam $a_1$ and the deflected light beam $a_c$ of filter $A_{EM}$
	$\alpha_B$	angle between the incident light beam $b_1$ and the deflected light beam $b_2$ of filter $B_{EM}$
	E	image plane
	$\overline{P_A P_B}$	distance (pixel shift) between the image points impinging on the image plane E

The light beams  $a_1$  and  $b_1$  impinge on the emission filters  $A_{EM}$  and  $B_{EM}$  of the corresponding filter sets A and B. The beam is deflected in more or less opposite directions because of the existing wedge angle of the filters depending on

the installed position ( $a_2$  and  $b_2$  are greatly exaggerated in the drawing in order to illustrate the process). Therefore, the image points impinging on the image plane E do not lie exactly one above the other, but are offset relative to one another by the pixel shift. Even with the close tolerances of the filters sets by Carl Zeiss with a slight image offset, this offset still occurs to a slight extent.

#### OBJECT AND SUMMARY OF THE INVENTION

The object of the invention is to overcome the slight image offsets caused by different wedge angles of emission filters and color splitters in a fluorescence microscope.

In accordance with the invention, a fluorescence microscope with blocking filters for a portion of the light emitted by a specimen are marked with respect to the orientation of their wedge angle.

#### BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings:

Fig. 1 is a schematic illustration of a fluorescence microscope;

Fig. 2 is a diagrammatic view of how the different wedge angles of the emission filters and of the color splitters cause a slight image offset; and

Fig. 3 is a diagrammatic view of how the filters are aligned with one another with respect to their wedge angle.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

According to the invention, as is shown in Fig. 3, the filters are aligned with one another with respect to their wedge angle. The filters are measured and marked by the microscope manufacturer beforehand with respect to wedge angle and orientation, for example, in an autocollimator, e.g., by means of a line S on the side which can be arranged, e.g., on the side located opposite the deflecting direction through the wedge effect. When the filter is inserted into the respective filter module of the microscope, this filter module also has a marking which is made to

coincide with the marking on the filter. Identical orientation of the filters is ensured in this way.

After the emission filters  $A_{em}$  and  $B_{em}$  are swiveled in (see Fig. 1), the impinging light beams  $a_1$  and  $b_1$  are deflected in the same direction ( $a_2$  and  $b_2$ ). In this way, the pixel shift which exists to a slight extent in any case is minimized or, ideally, compensated (pixel shift  $\overline{P_A P_B'}$ ).

In this connection, the wedge angles can also be determined on the part of the manufacturer and filters with identical wedge angles can be marked and correlated by the user

While the foregoing description and drawings represent the present invention, it will be obvious to those skilled in the art that various changes may be made therein without departing from the true spirit and scope of the present invention.